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1: Vaccine 1995 Jun;13(9):803-10

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Immunoregulatory role of H-2 and intra-H-2 alleles on antibody responses to recombinant preparations of B-subunits of Escherichia coli heat-labile enterotoxin (rEtxB) and cholera toxin (rCtxB).

Nashar TO, Hirst TR.

Research School of Biosciences, University of Kent at Canterbury, UK.

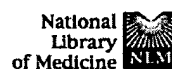
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The immunoregulatory role of H-2 and intra-H-2 alleles on antibody responses to recombinant preparations of B-subunits of Escherichia coli heat-labile enterotoxin (rEtxB) and cholera toxin (rCtxB) is reported. Oral delivery of rEtxB to congenic mice of several different H-2 haplotypes resulted in H-2 dependent serum IgG responses (H-2d > H-2b = H-2q > H-2a > H-2k) and a similar spectrum of intestinal IgA responses in those strains tested. Responses to rEtxB and rCtxB were found to be differentially modulated by the H-2 locus, with significant differential effects in H-2b and H-2d congenic strains (H-2d > H-2b for rEtxB; H-2b > H-2d for rCtxB). Additionally, it was found that when rEtxB was fed to mice previously primed (orally) with either rEtxB or rCtxB only those mice primed with rEtxB exhibited a booster response. A second booster immunisation with rEtxB in rCtxB-primed mice produced an H-2 dependent spectrum of responses characteristic of those elicited by rEtxB, with the antibodies predominantly directed against rEtxB and not rCtxB. These results indicate that the differential response to rEtxB and rCtxB is set at the T- and B-cell level. Also, immunoregulation of antibody responses to rEtxB by intra-H-2 I-E in mice transgenic for the entire IEka gene was investigated. No significant difference between responses in transgene-positive and -negative mice was found, suggesting that antigen presentation does not involve I-E, but occurs in the context of I-A. (ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 7483801 [PubMed - indexed for MEDLINE]

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1: Vaccine 1996 Jun;14(8):792-8

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Humoral and cellular immune responses in the murine respiratory tract following oral immunization with cholera toxin or *Escherichia coli* heat-labile enterotoxin.

Ruedl C, Rieser C, Kofler N, Wick G, Wolf H.

Institute for General and Experimental Pathology, Medical School, University of Innsbruck, Austria.

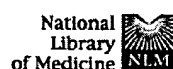
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Cholera toxin (CT) and *Escherichia coli* heat-labile enterotoxin (LT) are the strongest mucosal immunogens identified to date and are also good adjuvants when given orally together in combination with unrelated antigens. We used these potent immunogens to monitor local and systemic immune responses following oral immunization of BALB/c mice, and compared their action on the following: (a) immunoglobulin production rates (IgG, IgM and IgA) in mucosal inductive (Peyer's patches-PPs), effector (intestinal lamina propria-LP, respiratory tract) and systemic (spleen) sites; (b) analysis of systemic antigen-specific antibodies (IgG subclasses, IgA and IgE); (c) time monitoring of fecal anti-CT and anti-LT antibodies, and (d) in vivo relevance of interleukin-6 (IL-6) to mucosal responses. Both mucosal immunogens elicited specific antibody responses (IgA, IgG) not only in the gastrointestinal tract (PP's and intestinal LP), but also in the respiratory tract and spleens of orally immunized mice. These mucosal responses were accompanied by elevated secretion of IL-6 in all investigated tissues, indicating involvement of this cytokine in B-cell maturation processes. Furthermore, oral immunization with CT and LT induced elevated serum titers of IgG1 followed by IgG2a, IgG2b, IgG3 and IgA, while high antigen-specific IgA and IgG1 responses were found in fecal extracts. These findings illustrate the action of orally administered CT and LT, respectively, on several humoral and cellular immune responses not only at the gastrointestinal tract, the application site, but also in distant mucosal effector sites such as the respiratory tract. These data suggest the potential use of these mucosal adjuvants in oral immunization strategies to improve the local immune response in remote mucosal tissues, in accordance with the concept of a common mucosa-associated immune system.

PMID: 8817827 [PubMed - indexed for MEDLINE]

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1: Immunology 1997 Jul;91(3):361-8

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Differential regulation of IFN-gamma, IL-10 and inducible nitric oxide synthase in human T cells by cyclic AMP-dependent signal transduction pathway.

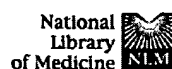
Benbernou N, Esnault S, Shin HC, Fekkar H, Guenounou M.

Laboratoire d'Immunologie, Faculte de Pharmacie de Reims, France.

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Expression of cytokines by T lymphocytes is a highly balanced process, involving stimulatory and inhibitory intracellular signalling pathways. In the present work, we attempted to clarify the role of cAMP on interferon-gamma (IFN-gamma), interleukin (IL)-10, IL-4 and IL-13 expression as well as on the inducible nitric oxide synthase (iNOS) expression. Treatment of phytohaemagglutinin (PHA)/phorbol 12-myristate 13-acetate (PMA)-activated Jurkat cells with either dibutyryl-cyclic adenosine monophosphate (cAMP) or pentoxifylline induced a strong inhibition of IFN-gamma mRNA expression as measured by reverse transcription (RT)-polymerase chain reaction (PCR), without affecting IL-10 expression. Both cholera toxin and prostaglandin E2 (PGE2) induced a strong inhibition of IFN-gamma mRNA expression, whereas IL-10 mRNA expression was significantly enhanced. This differential regulation of IFN-gamma and IL-10 expression was related to intracellular cAMP concentration. IL-13 and IL-4 mRNA expressions were not inhibited. We developed a new method based on immunofluorescence for intracellular cytokine detection followed by optical and computerized image processing, and our results showed that IFN-gamma protein was strongly inhibited when cells were treated with PGE2 or dibutyryl (db)-cAMP, whereas IL-10 protein was enhanced. This suggests that cAMP exerts its action at both the transcriptional and protein levels. iNOS mRNA expression was markedly elevated in the presence of PGE2. The generation of nitric oxide using sodium nitroprusside (SNP) induced a dramatic decrease of IFN-gamma, while IL-10 was enhanced; and conversely the inhibition of iNOS activity using 1-NG-monomethyl arginine (1-NMMA) induced a clear inhibition of IL-10 and IL-4, while IFN-gamma was enhanced. These results provide evidence that the protein kinase A (PKA) activation pathway plays a prominent role in the balance between the type 1 and type 2 cytokine profile in PHA/PMA-activated Jurkat cells. Data also suggest that iNOS expression is under the control of PKA activation, and that NO seems to be able to assume the polarization of activated T cells to the type 2 profile.

PMID: 9301524 [PubMed - indexed for MEDLINE]



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1: Vaccine 1999 Aug 20;18(1-2):38-49

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The role of cAMP in mucosal adjuvanticity of *Escherichia coli* heat-labile enterotoxin (LT).

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Services**Cheng E, Cardenas-Freytag L, Clements JD.**

Department of Microbiology and Immunology, Tulane University Medical Center, New Orleans, LA 70112-2699, USA.

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Heat-labile enterotoxin (LT) produced by enterotoxigenic *Escherichia coli* (ETEC) and cholera toxin (CT) produced by *Vibrio cholerae* have been shown to function as potent mucosal adjuvants. A number of studies have examined the effects of different mutations at either the active site or the protease site of LT and CT and the influence of those mutations on toxicity and adjuvanticity. However, different observations reported by various groups using a variety of animal models with different antigens or different routes of immunization have provided contradictory findings and evoked many questions regarding the underlying mechanisms of mucosal adjuvanticity of LT and CT. In this study, the role of cAMP in mucosal adjuvanticity was examined by comparing three LT active site mutants (S61F, A69G, E112K), a protease site mutant (R192G) and recombinant LT-B for toxicity, cAMP activity and mucosal adjuvanticity using tetanus toxoid (TT) as a model antigen. While all mutants examined showed reduced toxicity, the effects of each mutation on its ability to function as an adjuvant varied. Following intranasal immunization, native LT as well as protease and active site mutants of LT induced serum anti-TT IgG and their responses were virtually indistinguishable from one another. In addition, LT-B was also able to enhance production of serum anti-TT IgG, though at a level significantly lower than that achieved by native LT and mutants. Following oral immunization, the best serum anti-TT IgG responses were obtained with native LT and mutants that retained the ability to induce accumulation of cAMP. Despite the nearly identical serum anti-TT IgG responses following intranasal immunization, there was a strong correlation between the ability to induce accumulation of cAMP in cultured Caco-2 cells and the ability to elicit production of antigen-specific Th1 or Th2 cytokines.

PMID: 10501233 [PubMed - indexed for MEDLINE]

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